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Novel Experimental Therapeutic and PET Imaging of Activated Macrophages in Rheumatoid Arthritis

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2018

document version

Publisher's PDF, also known as Version of record

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citation for published version (APA)

Chandrupatla, D. M. S. H. (2018). *Novel Experimental Therapeutic and PET Imaging of Activated Macrophages in Rheumatoid Arthritis*. [PhD-Thesis - Research and graduation internal, Vrije Universiteit Amsterdam].

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9.1. Summary

After specifying the outline of the thesis in **Chapter 1**, **Chapter 2** provides an overview of the chronic disease rheumatoid arthritis (RA), with a focus on the role of activated macrophages and the potential of the folate receptor beta (FR β) as a macrophage mediated imaging and therapeutic target.

Chapter 3 describes the modification of an experimental model of RA in rats with the aim to create sustained articular macrophage infiltration, so that it could be used for both PET imaging and monitoring response to (new) therapeutic agents for RA. Adjustments consisted of a slightly adapted immunization procedure, increased volume of the first locally induced arthritis with methylated bovine serum albumin (mBSA) in the right knee of rats and, finally, repeat injections of mBSA to sustain arthritis for a longer period of time to be able to demonstrate efficacy of (multiple) administrations. The contralateral knee served as an internal control for each individual rat. These adjustments led to a relatively mild animal model of RA (no major body weight loss or other impairments). Significant anti-mBSA serum levels, DTH and knee swelling were observed with sustained and prolonged macrophage infiltration especially after repeated mBSA injections. To demonstrate the feasibility of PET evaluation of arthritis, two PET tracers were used [^{18}F]FDG (as a non-specific PET tracer for inflammation) and (*R*)-[^{11}C]PK11195 (binding to the macrophage marker translocator protein, TSPO, up-regulated in activated macrophages). Both tracers showed increased accumulation in the arthritic knee as compared with the contralateral control knee, which reflected microscopic macrophage infiltration as demonstrated by immunohistochemistry. Interestingly, the contralateral knee showed some macrophage infiltration as well, although much less than the arthritic knee, being indicative of a systemic component of RA in this model.

In **Chapter 4**, this rat model of RA was used to investigate the feasibility of visualising articular inflammation with the macrophage PET tracer [^{18}F]fluoro-PEG-folate, which targets FR β . Moreover, in this study a systemic component of the inflammation was demonstrated in the liver and spleen of these rats. In addition, a first PET therapy response monitoring was performed during treatment of arthritic rats with MTX, the anchor drug in RA. After MTX treatment, [^{18}F]fluoro-PEG-folate PET images revealed attenuation of arthritis which was corroborated by markedly reduced macrophage infiltration in the arthritic knee of the rat. These studies were extended in **Chapter 5** with ex vivo tissue distribution studies of [^{18}F]fluoro-PEG-folate: sections of arthritic knees and multiple (macrophage residing) organs were analysed histologically and by immunohistochemistry using specific antibodies to rat macrophages, as well as FR β immunofluorescence measurements. It was demonstrated that MTX also reduced systemic inflammation in arthritic rats as confirmed by significant reductions in macrophage numbers in both spleen and liver of arthritic rats.

In **Chapter 6**, the arthritic rat model was employed to explore the potential anti-arthritic effects of a new agent, alkaline phosphatase (AP). AP functions as a gate-keeper of innate immune system responses by detoxifying (dephosphorylating) inflammation triggering moieties (e.g. ATP, ADP, LPS), released from endogenous and external sources. Administration of human recombinant AP was tested in both prophylactic

(before arthritis induction) and therapeutic (after arthritis induction) settings, the latter as single agent and in combination with MTX. Prophylactic and therapeutic schedules of single agent AP treatment, and combinations with MTX, were well tolerated. Both prophylactic and therapeutic AP administrations resulted in markedly reduced synovial macrophage infiltration in arthritic knees, comparable with MTX treatment effects. AP and MTX combinations slightly improved on single agent effects. [^{18}F]fluoro-PEG-folate PET scans and ex vivo tissue distribution studies confirmed the effects of AP and AP+MTX in reducing synovial macrophage infiltration. In addition to localized articular effects, AP also conveyed systemic anti-inflammatory effects by significant reductions in FR β -positive macrophages in liver and spleen of arthritic rats. Given these broad effects, single agent AP, or combined with MTX, deserves further evaluation as a new therapeutic modality in RA.

In **Chapter 7**, PET imaging was used to investigate targeting features and in vivo pharmacokinetics and pharmacodynamics of a new promising therapeutic agent, antibody fragment F8-mediated IL10 delivery (F8IL10), for RA. F8 binds to the extra-domain-A (ED-A) of fibronectin at sites of inflammation, allowing for local deposition of IL10. In a translational setting, radiolabelled F8-IL10 was injected in RA patients and in animals (with and without arthritis). Results in RA patients demonstrated clear targeting of radiolabelled F8-IL10 in the (sub)clinically inflamed joints. Remarkably, rapid clearance of F8-IL10 from the blood together with accumulation in liver, and to a lesser extent in spleen, were also noted. In addition, animal experiments were performed to elucidate the rapid uptake in liver (and spleen). These studies showed specific binding of F8-IL10 in liver and spleen of arthritic rats, which appeared to be due to increased fibronectin ED-A expression in these tissues as compared with similar tissues in healthy animals. Injection of a microdose of the PET tracer might result in increased (relative) uptake in liver and spleen upon first passage through these organs, but this remains to be proven in future studies. This translational study demonstrated the value of in vivo PET-CT biodistribution studies of new and potential anti-rheumatic drugs.

Chapter 8 reports on flow cytometric expression profiling of FR β on monocyte subpopulations (classical, intermediate, non-classical monocytes) in blood of early (treatment naïve) and established RA patients, with healthy volunteers serving as controls. Moreover, using immunofluorescence studies, macrophage FR β expression was characterised in synovial tissue biopsies from RA patients with variable degrees of disease activity. These studies were accompanied with stainings for other macrophage markers (CD163, CD169 and CD206) to define FR β expression in relation to ‘M1’-type (pro-inflammatory) and ‘M2’-type (anti-inflammatory) macrophage polarizations. In early RA patients (i.e. early stage of disease), markedly elevated FR β expression levels were noted in intermediate and (pro-inflammatory) non-classical monocytes, which comprise 10-15% of the monocyte population in peripheral blood. Since FR β expression levels were not elevated in established RA patients and healthy volunteers, FR β may represent a marker for pro-inflammatory monocytes in early RA patients. In paired samples (blood and synovial fluid) from established RA patients, FR β expression was more prominent in monocytes/macrophages in synovial fluid as compared with blood. FR β expression was clearly increased in synovial tissue of RA patients with active systemic and local disease as compared with RA patients with milder disease activity and non-RA synovial tissue.

Co-expression studies of $\text{FR}\beta$ with other macrophage markers revealed co-expression with known M2-type macrophages (i.e. CD163 and CD206). However, $\text{FR}\beta$ co-expression was not exclusively with M2-type markers, as also co-expression with M1-like CD169 macrophages and triple staining of $\text{FR}\beta^+\text{CD169}^+\text{CD163}/\text{CD206}$ were observed.